SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL MATERIALS AND METHODS

Preparation of human amniotic membrane-derived mesenchymal stem cells (hAMSCs).

Placenta was donated by families after childbirth at Stanford Department of Obstetrics and Gynecology. Amniotic membrane was isolated, digested, and cultured as previously described[1].

Preparation of mesenchymal stem cell-derived induced pluripotent stem cells (iPSCs).

The monoclonal iPSCs lines were generated by transfection of hAMSCs with non-integrating Sendai virus (Thermo Fisher Scientific Inc., MA), which include *OCT3/4, SOX2, KLF4, L-MYC, LIN-28,* short-hairpin RNA for P53 and EBNA1 in chemically defined media. Reprogrammed hAMSCs were transferred onto mouse embryonic fibroblast feeder cells (Cat. A24903, Thermo Fisher Scientific) on 0.1% gelatin-coated plates at day 3 after transfection and cultured in Essential 8 pluripotent stem cell medium (E-8 media, Life Technologies, CA). At day 20 after transfection, nascent iPSC colonies were picked and cultured independently on matrigel-coated plates in E-8 medium. Pluripotency was confirmed through qPCR gene expression analysis of the pluripotency genes *Nanog, Oct3/4* and *Sox2* (data not shown).

Preparation and purification of iPSC-derived cardiomyocytes (iCMs).

iPSC monoclonal lines were differentiated into iCMs under chemically defined conditions using small molecules as previously described [2]. 85% confluent iPSCs were incubated with CHIR (6 μM, D0-2) and then with C59 (2 μM, D3-5) in basal iCM differentiation medium (Gibco® RPMI 1640 medium, GlutaMAXTM Supplement, Thermo Fisher Scientific) with B-27 Supplement Minus Insulin (Thermo Fisher). On day 6, the medium was replaced with iCM maintenance media (Gibco® RPMI 1640 medium, GlutaMAX™ Supplement, Thermo Fisher Scientific) with B-27 Supplement. Upon spontaneous contractility (days 12-16), cells were replated to new matrigel-coated plates and glucose-free iCM maintenance media for 48 hours for purification.

Nanoparticle tracking analysis (NTA)

Exosome size and quantity were characterized by NTA with a NanoSight LM20 (NanoSight, UK). Samples were loaded into the sample chamber with sterile syringes and imaged using a 640-nm laser. Three measurements of the same sample were performed at room temperature (continuously monitored and manually entered). Particle size and velocity in the fixed chamber was used in a proprietary software, NTA 3.1 (NanoSight), to determine the mean size and standard deviation (SD) values of the particles. To verify that the concentration readings were representative, samples were measured in three serial measurements and averaged per the software protocol. Furthermore, a second set of 3 measurements were done at half or double the concentration, depending on the signal-to-noise from the initial triplicate run. The measured concentrations would be compared. Per protocol, concentrations within 20% of each other were indicative of accurate concentration readings.

Patch clamp analysis

iCMs at days 15-20 were dissociated using TrypLE for 10 min, filtered through a 100 μM cell strainer (BD Biosciences, CA), counted with a Countess Cell Counter, plated as single cells (1×10^5 cells per well of a 24-well plate) on 8 mm no. 1 glass cover slips (Warner Instruments, CT) coated with Synthemax II-SC (625 ng/cm2) in CDM3 supplemented with 2 μM thiazovivin and allowed to attach for 72 hr, changing the medium every other day. Cells were then subjected to whole-cell patch clamp at 36-37°C, using an EPC-10 patch-clamp amplifier (HEKA, Germany) attached to a RC-26C recording chamber (Warner Instruments) and mounted onto the stage of an inverted microscope (Nikon, Japan). Sharp microelectrodes were fabricated from standard wall borosilicate glass capillary tubes (BF 100-50-10, Sutter Instruments) using a P-97 Sutter micropipette puller to generate electrodes with tip resistances between 50 MΩ and 70 MΩ when backfilled with 3 M KCl. Cell cultures were perfused with warm (35-37°C) Tyrode's solution consisting of 135 mM NaC1, 5.4 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 0.33 mM

NaH2PO4, 5 mM HEPES and 5 mM glucose; pH was adjusted to 7.4 with NaOH. Membrane potential measurements were made using the current clamp mode of the Multiclamp 700B amplifier after electrode potential offset and capacitance were neutralized. Data were acquired using PatchMaster software (HEKA) and digitized at 1.0 kHz. The following are the criteria used for classifying observed action potentials (APs) into ventricular-, atrial- and nodal-like human iPSC-derived cardiomyocytes. For ventricular-like, the criteria were a negative maximum diastolic membrane potential (<−50 mV), a rapid AP upstroke, a long plateau phase, AP amplitude > 90 mV and AP duration at 90% repolarization/AP duration at 50% repolarization (APD)90/APD50 < 1.4. For atrial-like, the criteria were an absence of a prominent plateau phase, a negative diastolic membrane potential (<−50 mV) and APD90/APD50 > 1.7. For Nodal-like, the criteria were a more positive MDP, a slower AP upstroke, a prominent phase 4 depolarization and APD90/APD50 between 1.4 and 1.7.

Immunogold labeling and transmission electron microscopy (TEM) of the exosomes

Approximately 10 x 10^9 iCM-Ex (as quantified by NTA) were incubated with mouse anti-CD9 antibody overnight at 4°C. Samples were diluted 1:10 in PBS and incubated with 1.4-nm nanogold-conjugated secondary anti-mouse antibody (Nanoprobes, NY) for one hour, followed by crosslinking with 1% glutaraldehyde for 15 minutes, both at room temperatures. Sample volumes were brought to 500 µL then filtered through Amicon Ultra 0.5 mL centrifugal filters, MWCO 100 kDA (Millipore Sigma, MO) for buffer exchange according to the manufacturer's recommendations. TEM grids (ultra-thin carbon film supported by Lacey carbon grid 300 mesh, Ted Pella, CA) were glow-discharged (PELCO easiglow) (Ted Pella), applying a current of 35 mA for 60 s. After immunogold labeling and chemical cross-linking, 3 µl of purified exosome samples were applied to the glow-discharged TEM grid. The sample was incubated on the grid for 60 s for absorption on the carbon, excess liquid was blotted (Whatman filter paper No. 1), followed by staining with a 1% phosphotungstic acid solution for 60 seconds, finally blotted and

dried. The negatively stained sample was mounted on a room-temperature side-entry holder and loaded into a Tecnai F20 TEM (FEI, OR) equipped with a K2 detector (Gatan, CA). The microscope was operated in low gain mode and images were acquired with a nominal magnification of 25,000 □ (corresponding to a pixel size of 1.52 Å / pixel) for a total of 8 seconds (200 ms per frame) with a total dose of 150 electrons / $Å^2$. Beam induced motion and stage drift was corrected by whole frame alignments using the MotionCor software tool, bandpass filtered (10 and 200 A) for visualization purposes and finally analyzed in ImageJ 1.49v (Wayne Rasband, NIH, MA).

Exosome miRNA Isolation

Total RNA was isolated from iCM-Ex using Total Exosome RNA and Protein Isolation Kit (Thermo Fisher Scientific) or from iCMs using miRNeasy Mini Kit (Qiagen, Germany). miRNA array was performed using Affymetrix® FlashTag™ Biotin HSR RNA Labeling Kits (Thermo Fisher Scientific), according to manufacturer's recommendations. Bioinformatics and pathway analyses were performed using Affymetrix® Expression Console (Thermo Fisher Scientific) and Transcriptome Analysis Console (Thermo Fisher Scientific), and Ingenuity Pathway Analysis (IPA, Qiagen).

Murine acute myocardial injury (MI) model

Animal care and interventions were done in accordance with the Laboratory Animal Welfare Act and Stanford University Administrative Panel on Laboratory Animal Care. All animals received humane care and treatment in accordance with the "Guide for the Care and Use of Laboratory Animals" (www.nap.edu/catalog/5140.html). Immunosuppressed female 80-100 day-old SCID-beige female mice (Charles River Laboratories, MA) were anesthetized in an isofluorane inhalational chamber and endotracheally intubated with a 20-gauge angiocatheter (Ethicon Endo-Surgery, NJ). Ventilation was maintained with a Harvard rodent ventilator (Harvard Apparatus, MA). Acute MI was created by ligation of the mid left anterior descending coronary artery

(LAD) through a left thoracotomy. A blinded surgeon injected 60 µl of iCM-exosomes (400 x 10^8) or iCMs (500,000) suspended in a 1:1 mixture of PBS and Matrigel into the myocardium at the border peri-infarct region (PIR): iCM-Ex (n=8), 2) iCMs (n=6), control (n=11).

Magnetic resonance imaging (MRI)

Cardiac MRI (Signa 3T HDx) (GE Healthcare, WI) was performed using a dedicated mouse coil (Rapid MR International, OH). Mice were imaged at weeks 2 and 4 after LAD ligation. Mice were anesthetized with 1-2% isoflurane at 2L/min oxygen and placed in the supine position. Electrocardiographic gating was obtained with two subcutaneous precordial leads and body temperature was monitored with a rectal probe during the entire scan (SA Instruments, NY). Left ventricular function was evaluated with electrocardiographically triggered Fast Spoiled Gradient-Recalled (FSPGR) sequence (TR 24ms, TE 10ms, FA 45, field of view (FOV) 6 cm^2 , matrix 256 × 256, slice gap 0mm, slice thickness 1 mm, NEX 4, 2 excitations, and 20 cardiac phases). Manganese-enhanced MRI (MEMRI) was performed using fast gradient echo-inversion recovery (FGRE-IR) sequence (TR 13ms, TE 6ms, FA 30°, FOV 4cm2 , matrix 256 × 256, slice gap 0mm, slice thickness 1mm, 2R-R acquisition, TI 300-500ms, and NEX 2) after 40 minutes with intraperitoneal (IP) injection of 0.7cc/kg of SeeMore (Eagle Vision Pharmaceutical, PA) prior to MEMRI acquisition 8, 9. The Mn2+-based contrast agent in MEMRI is taken up by L-type calcium channel to generate T1-shortening and positive signal, conferring high specificity to the viable myocardium. The images were analyzed offline using Osirix (Pixmeo, Switzerland) with manual contouring. Tracings of MEMRI was generated for each shortaxis slice and integrated to determine viable myocardial volumes in murine hearts. Percent MEMRI viable myocardial volume = (MEMRI enhancement volume × 100)/total left ventricular (LV) mass volume.

EdU Staining for Endogenous Proliferation

Mice were injected intraperitoneally with 0.1 mg EdU (Thermo Fisher Scientific) per gram body weight every 24 hours for 2 days prior to sacrifice, heart explantation, and preservation in OCT. 5 µm sections of the mid-ventricle were stained with Click-iT EdU Staining Kit (Thermo Fisher Scientific), cardiac troponin I (Thermo Fisher Scientific), and Hoescht 33342 per the manufacturers' recommendations. TNNI3 expression was used to locate the PIR. Images were acquired using a LSM 880 inverted confocal microscope (Zeiss, Germany). Number of cells expressing EdU-TNNI3 normalized to number of nuclei (representative of cell number).

***Ex vivo* mouse transcriptome analysis**

Mice were sacrificed 4 weeks post-MI. The PIR was isolated, homogenized, and RNA was isolated with miRNeasy® Mini Kit (Qiagen) according to manufacturers' recommendations. mRNA was arrayed using Clariom™ S Assays (Thermo Fisher) according to the manufacturers' recommendations. Bioinformatics and pathway analyses were performed using Affymetrix® Expression Console (Thermo Fisher Scientific) and Transcriptome Analysis Console (Thermo Fisher Scientific), and Ingenuity Pathway Analysis (IPA) (Qiagen). >2-fold differences in expression were considered significant.

***Ex vivo* RT-PCR analysis of the myocardial tissues**

RT-PCR was performed to verify significant gene expression as determined by the mouse transcriptome arrays. Total RNA, isolated using miRNeasy® Mini Kit (Qiagen) and verified with NanoDrop 2000 (Thermo Fisher Scientific), was reverse-transcribed with Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Relative gene expression was measured by Applied Biosystems™ StepOne Plus™ Real-Time PCR System (Thermo Fisher Scientific). GAPDH was used as an internal housekeeping gene. All reagents and instruments were used according to manufacturers' recommendations. Primer sequences can be found in Table S1.

Fibrosis Quantification

10 μm sections of the mid-ventricle were stained with Masson's Trichrome Stain Kit (Polysciences Inc., PA) per the manufacturer's recommendations. Images were acquired using a BZ-X710 microscope (Keyence, Japan). Fibrotic area (dark blue-stained tissue) in the left ventricle was normalized to the entire cross-sectional area.

Terminal deoxynucleotide Transferase dUTP Nick-End Labeling (TUNEL)

5 μm sections of the mid-ventricle were stained with with Click-iT TUNEL Staining Kit (Thermo Fisher Scientific), cardiac troponin I (Thermo Fisher Scientific), and Hoescht 33342 per the manufacturers' recommendations. Images were acquired using a LSM 880 inverted confocal microscope (Zeiss). Number of cells co-expressing TUNEL and TNNI3 was normalized to number of nuclei (representative of cell number).

Inhibition of Exosome Secretion

Manumycin A (Millipore Sigma) was reconstituted in DMSO. To fully inhibit exosome secretion, cells were pre-treated with 10 μM MA for 24 hours prior to assays.

Mitochondrial membrane integrity measurement of hypoxic cardiomyocytes

At the end of hypoxia exposure, JC-1 Mitochondrial Membrane Potential Probe (Cayman Chemicals, MI) was added to sample medium at concentration of 100 μL per 1 mL culture medium and incubated for 30 min at 37°C. Images were acquired using an BZ-X710 microscope (Keyence) and analyzed with ImageJ (NIH).

Transmission Electron Microscopy

On day 3 post-MI, mice were sacrificed and the hearts were perfused with Karnovsky's fixative (2% glutaraldehyde, 4% paraformaldehyde, in 0.1M sodium cacodylate pH 7.4). Tissue in the PIR were cut into approximately 1 mm^2 blocks and returned to fixative solution for 1 hour. The fix was replaced with cold/aqueous 1% osmium tetroxide and were then allowed to warm to room temperature (RT) for 2 hours rotating in a hood, washed 3X with ultrafiltered water, then

en bloc stained in 1% uranyl acetate at RT for two hours while rotating. Samples were then dehydrated in a series of ethanol (EtOH) washes for 30 minutes each at RT beginning at 50%, 70% ethanol then moved to 4ºC overnight. They were placed in cold 95% EtOH and allowed to warm to RT, changed to 100% 2X, then propylene oxide (PO) for 15 min. Samples are infiltrated with EMbed-812 resin (Thermo Fisher Scientific) mixed 1:2, 1:1, and 2:1 with PO for 2 hrs each with leaving samples in 2:1 resin to PO overnight rotating at RT in the hood. The samples are then placed into EMbed-812 for 2 to 4 hours then placed into molds and fresh resin, orientated and placed into 65ºC oven overnight. Images were acquired with a JEM1400 120kV Transmission Electron Microscope with a Gatan Orius CCD Camera (JEOL, MA). Autophagosomes were identified as double-membrane-bound vesicles containing organelle-like structures and quantified as the average of 20 random micrographs per animal.

RT-PCR

iCM RNA was isolated using miRNeasy Mini Kit (Qiagen) and reverse transcribed using High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific). Equal amounts of cDNA were loaded onto a 96-well plate for RT-PCR using in-house primers and Power SYBR Green PCR Master Mix (Thermo Fisher Scientific). Relative gene expression was measured by Applied Biosystems™ StepOne Plus™ Real-Time PCR System (Thermo Fisher Scientific). β-actin was used as an internal housekeeping gene. A list of primers can be found in Table S3.

SDS-PAGE and Immunoblot

Proteins were separated at 10% SDS-PAGE (Thermo Fisher Scientific) under reducing conditions and electrophoretically transferred onto polyvinylidine difluoride membranes (Biorad), followed by routine immunoblotting (IB). Antibodies can be found in Supplementary Table 3.

Table S1. Qualitative and quantitative criteria for classification of iCMs in three major cardiac cell types.

Nodal-like (N-like):
▪ Always generated spontaneous APs ▪ Exhibits a more depolarized MDP ▪ A prominent phase 4 depolarization ▪ Slower maximum rate of rise ▪ Shortest APD ▪ APD90/APD50: 1.4-1.7
Atrial-like (A-like):
▪ Triangular AP profile ▪ Absence of a prominent plateau phase ▪ A negative MDP (< -50 mV) ▪ More hyperpolarized MDP/RMP ▪ A faster rate of rise ▪ Intermediate APD ▪ APD90/APD50: > 1.7
Ventricular-like (V-like):
▪ A negative MDP (< -50 mV), ▪ A rapid AP upstroke, ▪ A long plateau phase, ▪ APA > 90 mV ▪ APD90/APD50: < 1.4

Table S2. Summary of action potential parameters of iCMs day 15-19 day of differentiation.

iCMs (n=23)	**% of cells (n)**	**Beating rate (bpm)**	**MDP (mV)**	**Overshoot (mV)**	**APA (mV)**	**APD90 (ms)**	**APD70 (ms)**	**APD50 (ms)**	**V_{max} (V/Sec)**
Ventricular-like	65 (15)	42.0 ± 4.3	-53.5 ± 1.0	39.3 ± 0.8	92.8 ± 1.2	342.4 ± 18.7	324.4 ± 17.5	302.1 ± 16.3	19.2 ± 0.9
Atrial-like	26 (06)	40.0 ± 1.1	-52.1 ± 1.6	38.4 ± 2.2	90.5 ± 3.3	259.1 ± 44.0	219.4 ± 37.9	177.4 ± 28.8	18.5 ± 1.7
Nodal-like	09 (02)	68.0 ± 1.9	-40.9 ± 1.2	27.0 ± 1.8	67.9 ± 3.0	236.4 ± 35.6	175.9 ± 47.6	140.6 ± 34.6	5.8 ± 1.8

BPM: Beat Per Minute

MDP: Maximal Diastolic Potential

APA: Action Potential Amplitude

APD_{50}: Action Potential Duration at 50% repolarization

APD_{70}: Action Potential Duration at 70% repolarization

APD_{90}: Action Potential Duration at 90% repolarization

Vmax: Upstroke Velocity

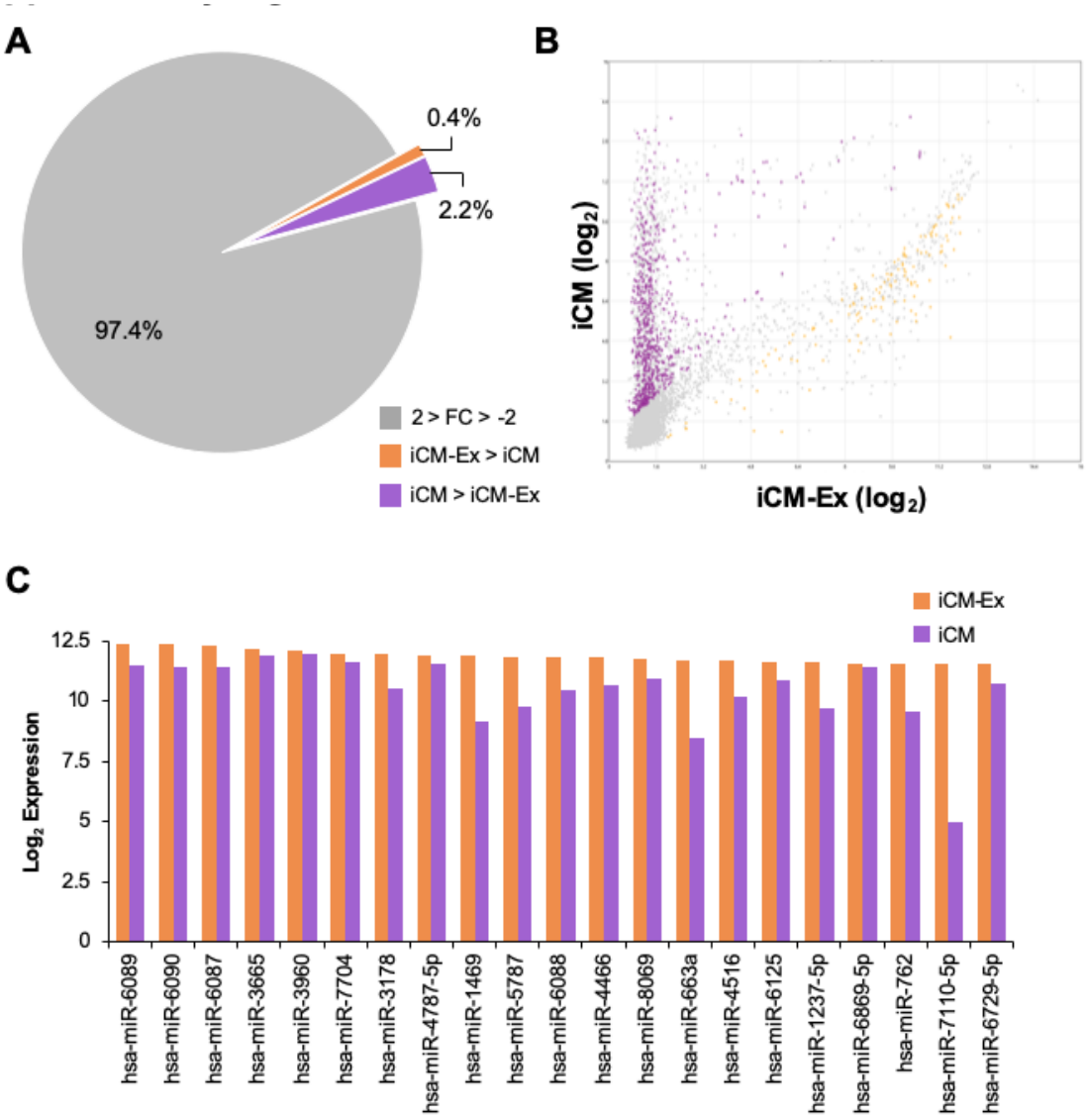
Table S3. Antibody Dilutions and Vendor Information.

Antibody	Dilution	Manufacturer	Catalog No.
A. Mouse anti-Human CD63	1:500	Abcam	Ab8219
B. Donkey anti-Mouse IgG (H+L), Alexa Fluor 700	1:10,000	Thermo Fisher Scientific	A10038
C. Mouse anti-Human CD9	1:500	Thermo Fisher Scientific	10626D
D. Nanogold anti-Mouse Fab' fragment	1:1,000	Nanoprobes	2002
E. Monoclonal Anti-α-Actinin (sarcomeric)	1:600	Millipore Sigma	A7811
F. Hoechst 33342, Trihydrochloride, Trihydrate	2 μg/mL	Thermo Fisher Scientific	H3570
G. Rabbit anti-Human, -Mouse, -Rat Troponin I	1:200	Thermo Fisher Scientific	701585
H. Rabbit anti-Human, -Mouse, -Rat p62	1:1,000	Cell Signaling Technology	5114
I. Rabbit anti-Human, -Mouse, -Rat LC3B	1:1,000	Cell Signaling Technology	2775
J. Monoclonal Anti-β-Actin	1:5,000	Millipore Sigma	A5441
K. Anti-Rabbit IgG, HRP Conjugate	1:5,000	Promega	W4011
L. Anti-Mouse IgG, HRP Conjugate	1:5,000	R&D	HAF007
M. Rabbit anti-Human, -Mouse, Rat Atg5	1:1,000	Cell Signaling Technology	2630

Table S4. Primer Sequences.

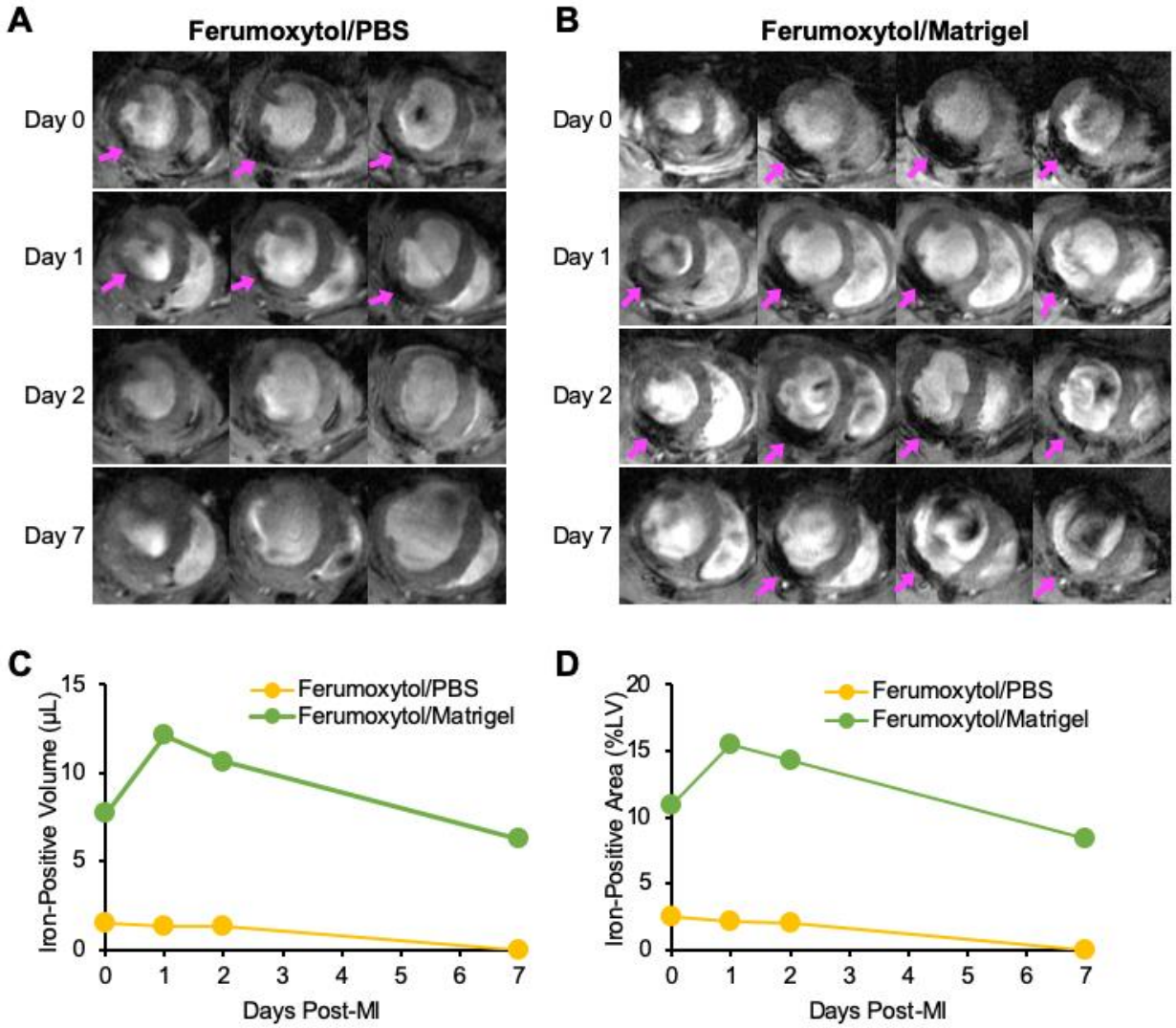
Species	Primer Name	Forward Sequence	Reverse Sequence
Mus Musculus	A. *BCL-2*	AGTTCGGTGGGGTCATGTGTG	CCAGGTATGCACCCAGAGTG
	B. *BCL-xL*	CACTGTGCGTGGAAAGCGTAG	CGACTGAAGAGTGAGCCCAG
	C. *GAPDH*	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA
	D. *mTOR*	AGAGGTCGGCACTCCACTAT	TGGCCAGGCTTCTGAACAAA
	E. *FKBP1*	AAGGGGTAGCCCAGATGAGT	CAGATCCACGTGCAGAGCTAA
	F. *RAPTOR*	TCTTATGGGACTCGGGGAGG	TGTGGTCTTCACCACATCCG
	G. *Bnip3*	ACGAACCCCACTTTGCAGTT	CCACCCAAGGTAATGGTGGA
	H. *Atg5*	GACGTTGGTAACTGACAAAGTGA	GCCATTTCAGTGGTGTGCCT
Homo sapiens	L. *β-actin*	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
	J. *Beclin-1*	GGGTTGCGGTTTTTCTGGGA	GTGTCTCGCCTTTCTCAACC
	K. *BNIP3*	AAGGCGTCTGACAACCTCC	GAGCTATGTTGCAAGCTCAGA

Figure S1. miRNA Profiles of iCMs and iCM-Ex.



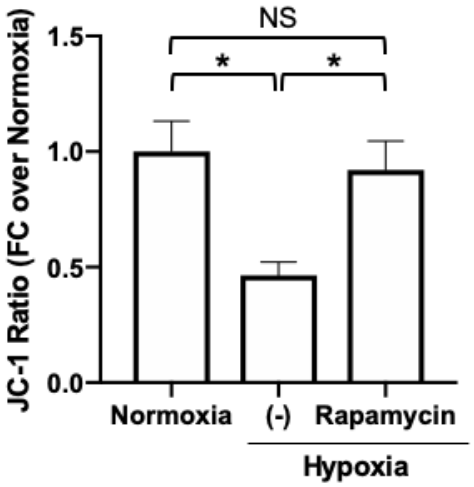
miRNA content of iCM and iCM-Ex were characterized by microarray. (A) The majority of miRNAs (97.4%) were expressed at similar levels (less than 2-fold change (FC)). (B) Relative expression levels of iCM and iCM-Ex miRNAs. (C) Top 20 highly expressed miRNAs in iCM-Ex and their respective expression in iCMs.

Figure S2. Matrigel enhances long-term retention of injected nanoparticles in myocardium.



Mice received acute MI and were injected with Ferumoxytol nanoparticles in the peri-infarct border. Prior to injection, Ferumoxytol was suspended in a solution of Matrigel/PBS or PBS alone. The mouse myocardium was imaged by cardiac MRI for 7 days post-MI. Hypointense signals denoted the presence of Ferumoxytol (pink arrows) in (A) mice injected with Ferumoxytol in PBS alone and (B) mice injected with Ferumoxytol in PBS with Matrigel. Ferumoxytol injected with Matrigel was detectable 7 days post-MI while Ferumoxytol injected with PBS alone failed to be detected by day 2. The hypointense signals generated by Ferumoxytol were quantified and plotted as (C) volume and (D) percentage of LV. Mice treated with Ferumoxytol/Matrigel showed greater iron-positive volume and iron-positive area than mice treated with Ferumoxytol/PBS.

Figure S3. Autophagy preserves mitochondrial membrane potential in hypoxic cardiomyocytes in vivo.



Mitochondrial membrane potential (MMP) was assessed by JC-1 fluorescent assay in three groups: normoxia, hypoxia untreated (-) control, hypoxia + rapamycin (scale bar = 100 μm). (B) MMP is significantly reduced in hypoxic (-) or MA-treated cells and restored by rapamycin treatment. Data are mean ± SEM of minimum n=3. *$p<0.05$.

SUPPLEMENTAL REFERENCES:

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2. Kim PJ, Mahmoudi M, Ge X, Matsuura Y, Toma I, Metzler S, Kooreman NG, Ramunas J, Holbrook C, McConnell MV, Blau H, Harnish P, Rulifson E, Yang PC. Direct evaluation of myocardial viability and stem cell engraftment demonstrates salvage of the injured myocardium. *Circ Res*. 2015;116:e40-50.